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DR. W. G. MACCALLUM, *President.*

Through an error on the part of the Editor, the closing discussion of Dr. Rouse upon his paper—"A Sarcoma transmitted by an Agent Separable from the Tumor Cells"—was omitted from Vol. XI, Nos. 1 and 2, and is therefore included in the present number.

Discussion:

A SARCOMA TRANSMITTED BY AN AGENT SEPARABLE FROM THE TUMOR CELLS.

PEYTON ROUS, M.D.

DR. ROUS replying, said with reference to Dr. Field's remarks on the permeability of Berkefield filters, that these filters are undoubtedly often faulty, but that the evidence for an agent separable from the sarcoma cells does not rest upon its filterability alone. As already mentioned, the growth has been pro-

duced by means of material dried to a powder and kept in that form for many days—a procedure which kills the cells of warm-blooded animals, including tumor-cells, as experiment has repeatedly shown. Leaving this aside, Dr. Rous could not agree with Dr. Field's contention that the size of *Bacillus prodigiosus* was such as to make it in any way comparable with tissue cells in filterability. New Berkefeld filters (N) were used and filtration hastened by means of the suction pump. Filtration was never continued for more than an hour, at the end of which time, as a rule, only 10 to 30 cc. of fluid had passed through.

It was suggested by Dr. Levin that the growth might be a multiple granuloma, and he mentioned the difficulty in differentiating morphologically some of these forms from the true tumors. But the diagnosis of the growth rests to an inconsiderable degree on its morphological characters, though these are characteristic of the sarcomata. Its method of dissemination and its behavior on transmission are of the sort which distinguish the true neoplasms. That a growth with such features should be produced by an agent separable from the cells is the important point. The name given it is immaterial.

According to Dr. Beebe the growth is in many respects unique among tumors, and he cited in evidence the early occurrence of metastasis from it. In the paper the fact was perhaps not made clear that the occurrence of metastasis is only early in point of actual time involved. When the malignancy of the growth and its rapid development at the site of inoculation are considered, it becomes evident that metastasis takes place at relatively the same period as from mammalian tumors. Not until the primary growth has been present some time, and has attained a good size, are secondaries ever found.

Though it is true, as Dr. Beebe pointed out, that no single transmissible neoplasm heretofore studied corresponds with the chicken tumor at all points in its behavior, yet the latter conforms absolutely to the type, to the composite picture of the malignant neoplasm derived from the study of many individual and often widely differing tumors. The chicken sarcoma is

perhaps unique in its close conformity to this picture at so many of its points, but in only one other respect, namely, in that there can be separated from its cells an agent which will produce the disease in new hosts.

When the sarcomatous tissue is heated to 55°C. for 15 min. it fails to cause the growth on implantation. Data on the resistance of the tumor cells and of the agent separable from them are now being collected.

HAEMANGIOMA INVOLVING THE SWEAT GLANDS.

JAMES CORSCADEN, M.D.

A tumor of typical warty vascular appearance was noticed at birth in a female child of four months. Within the few weeks preceding its removal there was very rapid growth. The tumor was in the lumbar region, five cm. from the mid-line and measured two by one cm. There was a similar growth the size of a pinhead on the buttock. On section the tumor appears as a very fine spongy network and is circumscribed.

Microscopically, there is a multilobular formation, the lobules being separated by dense connective tissue. The tumor is separated from the skin by dense connective tissue, and is bounded laterally and on its deep surface by fat. There is no distinct capsule. The tumor is composed of closely massed capillary spaces lined by large high endothelium in which occasional mitotic figures are seen. Near the skin the spaces are dilated and lined by flatter cells. Surrounding the endothelium is a thin layer of connective tissue, but no muscle. No transition from adjacent capillaries into those of the tumor is seen.

In most of the lobules are contained sweat glands intimately involved by the tumor tissue. There is no other connective tissue between the capillaries and the glands. No glands are seen in the interlobular connective tissue. The glands are never

compressed and seem to be distorted only by the volume of tissue between the tubules. They do not have the appearance of having been pushed aside by the growing tumor.

Interest in the specimen lies in the bearing of the contained glands on the theories of origin of the tumor. The relation of the vascular tissue to the glands makes it apparent that the growth is one of the blood vessels normally disposed about the sweat glands, there being in no section evidence of compression or displacement of the glands by a growing tumor.

Ribbert, in his injection experiments, claims independence of the tumor blood spaces from the adjacent capillaries, and explains the occasional finding of normal independent structures in the tumor mass by saying that these are remnants of the tissues pushed aside by the growing tumor.

Albrecht employed the term Hamartoma for those enlargements of normal tissues due to excess of growth, abnormal arrangement or irregular position. Under this head vascular naevus is included by Trappe. The evidence presented by the present tumor points to the latter theory.

Discussion:

Dr. E. Moschowitz: In examining sections of liver angiomata in the aged, Dr. Moschowitz noted that at the periphery of these tumors, bile ducts could be frequently found in the fibrous walls of the veins of capsule. It occurred to him that the growth of these angiomata could be, possibly at least, accounted for by the incorporation of the normal hepatic veins in Glisson's capsule by a process of atrophy of the intervening tissue—a process incident to old age. He assumed that the accompanying bile duct and artery became obliterated and that the cavernous structure peculiar to the older and interior portions of the angioma was due to the breaking down of the walls of the hepatic veins due to pressure. In order to determine this, he cut a number of these angiomata serially and found undoubted evidence that at the periphery, at least, the normal hepatic veins formed part of

the angioma. This observation, however, left the matter in the position of stale-mate, so to speak, because it was impossible to determine whether this phenomenon was really the result of liver atrophy, or the result of the normal growth of the angioma and consequent incorporation of the neighboring capsules of Glisson.

EXTENSIVE IRREGULAR NECROSIS OF THE LIVER IN AN INFANT.

C. W. FIELD, M.D., AND A. M. PAPPENHEIMER, M.D.

The liver which we wish to demonstrate was obtained at autopsy from an eighteen-day-old infant. The baby was one of twins born at eight months. Labor was simple, the puerperium uncomplicated.

The infant was well until the sixth day, when it refused the breast, and had to be fed with a medicine dropper. On the following day, it became slightly icteric and oedema developed about the feet. The oedema during the following days decreased; then reappeared, extending gradually to above the navel. There was also transient oedema of the face. The icterus diminished gradually, and disappeared entirely before death, but spasmodic twitchings developed and the child passed into coma, in which it died on the twelfth day of its illness.

The twin child was perfectly well until the seventh day. Then it too suddenly became unable to nurse, and developed oedema, and progressive icterus. A diminution in the amount of urine was noted, but no examination was made. The child died after four days and no autopsy was obtained.

The autopsy on the first child, which was held eighteen hours post-mortem, showed besides the lesions in the liver, double broncho-pneumonia and suppurative inflammation of the left mastoid. The appearance of the liver was striking. The organ

was about normal in size, the surface was smooth, the edge sharp, the consistence somewhat firmer than normal. The whole organ, both on the surface and on the cut section, showed a peculiar mottling—irregular areas of a greenish gray, slightly translucent appearance, alternating with reddish and yellowish red areas. The grayish areas, which were found subsequently to represent intact liver tissue, were slightly more prominent above the surface than the reddish portions.

They were sharply circumscribed, and on close inspection it could be seen that they were in relation to, and formed irregular mantles about, the larger portal spaces.

The mottled appearance observed in the gross is even more striking and distinct in the stained sections. Here one may discern sharply demarcated, irregular islands staining more deeply than the surrounding tissue and corresponding, under the microscope, to areas of well preserved liver cells. By far the greater portion of the liver, however, is composed of liver cells which have undergone regressive changes.

In sections stained in hematoxylon-eosin, the degenerated areas show no trace of lobular structure. The individual cells no longer show a columnar arrangement but are irregularly disposed and separated one from another. The degenerated cells are distinctly smaller than the normal cells. The protoplasmic outline is often indefinite. The cytoplasm of the affected cells does not take up the hematoxylon but shows a strong affinity for the eosin. With the immersion, the protoplasm has a foamy, vacuolated structure and in frozen fixed sections the cells are found filled with smaller and larger droplets staining bright orange red, with Sudan III. Nowhere, however, are there seen larger globules completely filling the cells and compressing the nucleus.

When treated by the Ciaccio method for the demonstration of the more complex lipoids, the degenerated cells show numerous droplets staining a dirty brownish yellow with Sudan III. Free flat globules are also found in small numbers within the capillaries. The nuclei are shrunken in comparison with that

of the normal cells, approximately not more than half the size. They are circular in outline and stain densely and diffusely. Occasionally they are in the form of irregular, pycnotic fragments lying at the periphery of the cells; occasionally also signet and crescent forms are seen. Some of the cells appear to have lost their nucleus altogether, although this may be due to the fact that the nucleus lies without the plane of the section.

The islands of unaffected liver cells form irregular mantles about the larger portal spaces and are also preserved in places as a thin layer just beneath the serosa. All the portal spaces, however, are not surrounded by normal liver cells. In general the islands of normal cells preserve their characteristic lobular structure and radial arrangement about the central vein. The individual cells are approximately of normal size, showing no hypertrophy when compared with cells from the normal liver of an infant of approximately the same age.

No fat globules or glycogen are present, but in the Ciaccio preparation many of the normal cells show a diffuse reddish brown discoloration. The capillaries in the normal areas are distended with well preserved blood cells. Here and there are found small groups of cells which under the immersion can be identified as normoblasts and megaloblasts. Large cells with a vesicular nucleus and somewhat more basophilic cells, probably representing precursors of the megaloblasts, are also found. In a few of the megaloblasts, karyokinetic figures were seen. These erythoblastic centers, which were studied in Giemsa-stained sections, are seen not only in the capillaries of the normal liver tissue but also in the degenerated areas. Moreover, nucleated red cells of all types are present in unusual numbers throughout the liver. There is, therefore, an active hemopoesis, apparently unaffected by the degeneration of the parenchymal elements.

There is very little fibrosis or evidence of active connective tissue hyperplasia. Some of the smaller vessels in the periphery of the lobules are surrounded by apparently actively growing fibroblasts, and in the neighborhood of the large portal trunks the connective tissue of Glisson's capsule is somewhat more

abundant than normal. With Bielschowsky's stain no increase in the intralobular connective tissue is apparent either in the normal or the degenerated regions.

There is in many places evidence of regenerative activity on the part of the smaller bile ducts. From the periphery of the lobules we see small ingrowths of cells with dark staining protoplasm containing various numbers of irregularly-sized vesicular nuclei, some of which are in active karyokinesis. Three mitotic figures were seen in a single mass of cells. The direct connection of these cell masses with the bile ducts in the portal spaces and at the periphery of the lobules can in some places be demonstrated in serial sections. The bile ducts give off lateral off-shoots which soon lose their lumen, branch, and penetrate the periphery of the lobules for a short distance. The cells are no longer cylindrical but irregularly polygonal, and tend to lie in double rows, though the terminations of the sprouts may be three or four cells in thickness.

Owing to the methods of fixation employed, no bile can be demonstrated in these new formed cells; neither do they contain fat in appreciable amount, or glycogen. The efforts at regeneration displayed by the intact liver cells are negligeable. A rare mitosis is found, but there is no reduplication of the cells of the liver cords, no loss of the radial arrangement, no formation of large multinuclear cells and no ingrowth of new formed cells into the degenerated lobules. This is probably due to the short course of the disease, affording no time for the development of the adenomatous hyperplasia described by Meder,[1] Marchand,[2] Stroebe,[3] MacCallum[4] and others in long standing cases of liver atrophy. Regeneration, in this case, therefore, is practically limited to the solid sprouts derived from the interlobular bile passages. Active and conspicuous as is this proliferation, it is not probable that it has in this case led to the regeneration of functional liver cells. The terminal solid buds resemble liver cells in the appearance of their nuclei, but the cells do not assume a characteristic polygonal shape of normal liver cells, nor do they enter into a characteristic arrangement with the persistent cap-

illaries. Furthermore, regressive changes may occasionally be noted in the nuclei of the terminal cells of the sprouts, showing that some at least of the new formed cells are short lived.

Very striking and significant is the absence of acute inflammatory changes. There is no accumulation of small, round cells in the portal spaces, no infiltration of polynuclears. Neither can there be observed the phagocytosis of necrotic cell detritus by the desquamated endothelial cells of the capillaries as described in cases of focal necrosis by Mallory. These features are opposed to the idea of a direct bacterial causation for the necrosis.

As in so many of the reported cases of acute atrophy of the liver, we are quite in the dark as to the real cause of the lesion. The finding of slight cirrhotic changes in the pancreas, together with rudimentary islands of Langerhans (some of which apparently show a direct connection with the pancreatic ducts) suggested syphilis as a possible factor. Syphilis has been brought into relation with acute yellow atrophy by a number of observers. Meder in 1895 was able to collect twenty such cases.

In the case here reported, no spirochetae were found in Levaditi sections from all the principal organs. This fact, together with the absence of other characteristic syphilitic lesions, seems sufficient to exclude syphilis as a positive causative factor.

Since attention has been called to necrotic lesions in the liver, occuring as a sequel to chloroform narcosis, it might be suspected that chloroform administered to the mother during labor might account for the lesion. We have, however, ascertained that no chloroform was used in this case. No cultures were made at the time of autopsy, so that we cannot say whether or not the liver lesion is a manifestation of a general bacterial infection. The absence of micro-organisms in the necrotic areas in sections stained by Gram, methylene-blue and Giemsa, as well as the lack of inflammatory reactive changes, seem against this possibility. The lesion represents evidently what we are accustomed to call a toxic necrosis; whether due to bacterial or metabolic toxins it is not possible to say.

Our study of the literature is still incomplete, but we have

thus far been able to find but one reported case of extensive necrosis of the liver occuring in young infants. This is the case published by Aufrecht[5] in 1896. The mother was a girl 19 years of age, primipara. The labor was normal After twenty-four hours there was noticed in the child swelling about the feet, which on the second day extended above the symphysis. The swelling is described as hard, doughy and could be pitted on pressure. On the second day, also, there developed slight general icterus; the urine contained a small amount of albumin and "leucin spheres." On the third day the oedema extended to the navel and punctiform hemorrhages appeared on the left leg. The icterus increased. Death took place on the sixth day. At the autopsy, sixteen hours after death, aside from scattered petechial hemorrhages in the serous membranes, the lesions were confined to the liver. The organ is described as weighing 100 g., was firm, bluish black in color and showed no acinal markings. Microscopically there was complete loss of the lobular structure. The nuclei were shrunken and stained poorly. The cells were vacuolated. There was extensive hemorrhagic extravasation. Many bacilli were found in the capillaries.

Although the report of this case is somewhat incomplete, there seems to be a resemblance in the clinical history as well as the pathological findings.

Todt[6] in 1903 collected from the literature thirty-two cases of acute yellow atrophy in infants and young children. None of these, with the exception of the case above quoted, occurred in the new-born; almost all of them were similar clinically and pathologically to cases occurring in adult life. In conclusion, we wish to call attention to the resemblance between the lesions in the liver of our case, and those produced in dogs by Pearce[7] by injection of large doses of hemolytic serum. As is well-known, he attributed the necrosis in his experimental lesions, to "obstructive congestion of the capillaries and the smaller branches of the portal vein by fused masses of red cells." In our case, there was absolutely no histological evidence of such a condition, and we have no basis for considering the lesion as the result of infarction.

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[4]MACCALLUM, W. G.: *Johns Hopkins Mospital Reports*, Vol. 10, p. 69.
[5]AUFRECHT: *Ctrllblt. f. Inn. Med.* No. 11, 1896.
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Discussion:

DR. PAPPENHEIMER: I think one of the peculiar faatures, which Dr. Field has not emphasized, is that there was no jaundice at the time of death, and only a slight amount during the course of the disease. The only explanation that we have been able to make for this curious fact is that in the degenerated portion, the destruction of the cells was so complete that there was practically no bile produced, while in the intact liver cells which presumably have their conducting mechanism intact, there was no reason for a stasis of bile. I believe there have been a few cases of acute atrophy of the liver in adults in which jaundice has not been marked.

ON ISO-AGGLUTINATION IN DOG'S BLOOD.

DRS. OTTENBERG, S. S. FRIEDMAN, AND D. J. KALISKI.

Recently two of us reported that normal iso-agglutination could be detected in the blood of rabbits and in the blood of steers.* We desire now to report one additional fact, viz.: the occurrence of iso-agglutination between the bloods of normal dogs.

Iso-agglutination of human blood has been known for about ten years. Ever since Landsteiner's work it has been known that all human beings can be divided sharply into four groups accord-

*Journal Experimental Medicine, May, 1911.

ing to the agglutinative power of their blood serum for each other's blood cells. There are two iso-agglutinins and two corresponding iso-agglutinable substances. One group of human beings has in the blood serum both agglutinins, and in the blood cells no agglutinable substances (that is, the cells are not agglutinable). Another group of persons has the first agglutinin and the second agglutinable substance. The third group has the second agglutinin and the first agglutinable substance. The fourth group has both agglutinable substances and no agglutinin. These peculiarities are a permanent characteristic of the blood of each individual. Before this society, in 1908, in a paper with Dr. Epstein, I announced my belief that these characteristics were hereditary and were inherited according to Mendel's law. At that time I had examined only five families and could not definitely prove the point. Recently von Dungern and Hirschfeld have proved that human iso-agglutinable substance is hereditary according to Mendel's law. Von Dungern has also shown by the method of absorption that many of the hetero-agglutinins of animals' serum can be identified with one or the other of the human iso-aglutinins, and that many of the hetero-agglutinable substances of animals' red cells are closely similar to the iso-agglutinable substances of human blood. Actual iso-agglutination in lower animals has been overlooked, however, probably because most of the agglutinations are very weak and require a special technique for their demonstration.

In a previous study on the quantitative relationships of agglutinative serum and agglutinable red cells it was found that an excess of cells absorbed a small amount of agglutinin without agglutination being visible at all. Therefore in the present studies the method used was that of mixing very large proportions of serum with very small proportions of red blood cells.

While natural iso-agglutinins had not been described before our work, immune iso-agglutinins had been developed in dogs by von Dungern and Hirschfeld. We have repeated their work and have developed immune iso-agglutinins in two out of six dogs injected. The mutual agglutinations of these six dogs are shown

in chart I. Whether these immune agglutinins are exaggerations of the natural agglutinins which (with an improved technique) we subsequently discovered, we cannot state at present.

CHART I.

"Immunized" Dogs.

		SERA.					
		A	B	C	D	E	F
CELLS	B	—	—	—	+	—	—
	C	—	—	—	+	—	—
	D	—	—	—	—	—	—
	E	—	—	—	+	—	—
	F	—	—	—	—	—	—

In looking for normal iso-agglutination between the bloods of different dogs we were at first unsuccessful and only succeeded when we adopted for our technique the mixing of fifteen parts of serum with one part of defibrinated blood. (The ease with which dogs' cells laked in normal saline solution was one of the causes of failure in the early experiments.) The mixtures were made in capillary pipettes of two to five milimeters diameter, using a modified Wright technique (as described by Epstein and Ottenberg, Archives of Internal Medicine, May, 1905). The reason for the use of this technique was that the work could be done with small quantities of blood, and particularly that in the thin layer of dilute cell-suspension in a small pipette it was possible to see fine agglutination which escaped observation in ordinary test tubes.

There are many precautions that are necessary to get constant results. The glass tubes must be thoroughly cleaned and washed in distilled water, and dried. After the mixtures are made the open ends of the pipettes are sealed with paraffine to prevent drying. The mixtures are generally observed for one hour at room temperature. At the end of this time with dogs' blood, agglutination when it occurs can usually be seen. A column of fluid about one inch long is found most convenient, and it is important to have the air bubbles (which by rotation of the tube are used as mixers) of a uniform, rather small, size. All the tests reported here were made in duplicate, that is, two of us

making independent readings found practically the same results. As stated above, the agglutination between the bloods of untreated dogs is feeble as compared with the iso-agglutination of human bloods or of immunised dogs. By thorough mixing the clumps can be made to disappear, but generally they form again if the mixture is allowed to stand.

Ten or twelve dogs were bled at a time, and their bloods tested against each other at once. As can be seen in the chart, the agglutinations which occur admit of a certain grouping, but, as in the case of rabbits' bloods, there are irregular agglutinations which do not fit well in the classification, and the groupings can therefore be regarded as only tentative. The one definite division of the bloods is into those whose serum is non-agglutinative and those whose serum agglutinates some other dog's cells. Agglutinability of the cells seems to be irregular. In general those animals whose sera agglutinate the cells of most of the other dogs have cells which are agglutinated by the sera of only a small number of the other dogs—and vice versa. Auto-agglutination was never observed, though it was tested for regularly.

CHART II.*

Dogs March 29

CELLS	SERA. 1	6	8	11	4	9	10	2	3	5	12
1	—										
6	+										
8											
11					+						
4	+	+	+	+		+	+				
9	+	+	+	+			+	—			
10	+				+						
2	+				+	—					
3	+	+	+	+							
5	+	+	+	+							
12	+	+	+	+	+						

* The heavily shaded lines represent a tentative grouping, although the authors do not believe that sharply defined groups, such as are found in human beings, exist in either dogs or rabbits.

CHART III.

Dogs April 22

SERA.

CELLS	11	18	8	13	17	4	2	15	16	5	4	14
11				+		+			–			
18	+					–				–		
8	+											
13						–	—	—	–	—	—	—
17							—	—	—	—	–	–
4	–	–					—	—	—	–	—	—
2						+	—	—	—	—	—	—
15					+	+	—	—		—	—	—
16						+	—	—	—	—	—	
5	+	+	–				—	—	—	—	—	—
9	+	+	+				—	—	—	—	—	—
14	+					+	—	—	—	—	—	—

As to the permanence of these peculiarities of dogs we can say little at present. Most of the tests were repeated with blood obtained after two or three days and were found to be unchanged. In a series of dogs (chart 3), examined on April 22 were six dogs which had been examined on March 29 (chart 2). In the meantime several of the dogs had been used in various transfusion experiments. It will be seen by direct comparison of the record of the six surviving dogs (chart 4), that only two new inter-agglutinations appeared, and only one of the former agglutinations was lost, out of thirty combinations. Whether these changes were due to the treatment which the dogs received, we are not at present prepared to say.

CHART IV.

Six Surviving Dogs
On April 22nd as compared with same dogs on March 29*

SERA.

(March 29th)

CELLS	8	11	4	9	2	5
8	—	—	—	—	—	—
11	—	—	+	—	—	—
4	+	+	—	+	—	—
9	+	+	—	—	—	—
2	—	—	+	—	—	—
5	+	+	—	—	—	—

(April 22nd)

CELLS	8	11	4	9	2	5
8	—	+	—	—	—	—
11	—	—	+	—	—	—
4	—	+	—	—	—	—
9	+	+	—	—	—	—
2	—	—	+	—	—	—
5	+	+	—	—	—	—

* Several of the dogs had been used in transfusion experiments in the interim.

Discussion:

DR. FIELD: I must say very frankly that I would not trust the results obtained with such a method. In working with agglutinins, as with all immune bodies, one cannot be too careful. In the first place, in agglutination, the quality of the glass makes a great deal of difference. Unless one used glass tubes of the very best quality, which had been washed under known conditions most carefully and tested, one could not accept the results at all, because one may get curious results if the technique is not of the best.

Dr. Levin: Dr. Ottenberg's paper presents the results of a comparative study, and the faults of the technique, if there are any, are identical for all sets of experiments. The errors would consequently be neutralized on the analysis of the results.

If I understand Dr. Ottenberg correctly, the presence of agglutination is not constant in the dogs. The phenomenon may be present at a certain time and then disappear.

I should like to know whether there is a method by the aid of which we can change the phenomenon at will. For instance, if two dogs were tested, one immediately after and the other before a meal, whether the property of iso-agglutination of the serum would be different.

The second question is whether the blood serum of a dog possesses the property of homo-agglutination. The study of this property as indicating the inter-relationship between cells and bloodserum of the same animal, may be of some aid in the investigation of the pathogenesis of malignant tumors.

Dr. Ottenberg: In regard to Dr. Field's criticism, there is a certain amount of truth in what he says. Notice, however, in regard to these experiments that they have all been repeated with substantially the same results. Some of the irregularities may have been due to such causes as he suggests. The method is not ideal.

I have tried out the method pretty thoroughly in human iso-agglutination and hemolysis tests, of which we have done many hundreds in the past three years. With this method the results have always agreed with the well known peculiarities of human iso-agglutination, and when hemolysis occurred it could always be confirmed by repetition of the test. In these agglutinations in the blood of various animals, generally far more delicate than in the blood of human beings, we probably do make an occasional mistake. But on the whole the tests come out quite constant, and they control one another, so that we feel confident that the results are all right.

With regard to Dr. Levine's first question, auto-agglutination has been stated to occur in pernicious anemia. I have often

looked for it myself, but have never found it. Probably what has been called agglutination in these cases is simply the clumpy appearance of any very anemic blood as it runs from the needle prick.

Landsteiner has indeed described auto-agglutination of normal human cells, but the highly artificial conditions of his experiment, which included bringing the mixtures down to the freezing point, render it highly improbable that the phenomenon which he observed have any relation to ordinary agglutination. As Landsteiner himself points out, auto-agglutinins, even if formed, would be extremely difficult, if not impossible to demonstrate, because being always in the presence of a large number of susceptible cells, the agglutinins would be absorbed as rapidly as formed.

With regard to Dr. Levine's second question, the only methods that I know of changing these properties in the living animal are by immunisation or by absorption. Immunisation I have already referred to. I recently saw a human blood transfusion which illustrated this absorption of agglutinin *in vivo*. Before the transfusion the patient's serum was agglutinative to the donor's cells, but not the donor's serum to the patient's cells. Serum obtained from the patient after the transfusion no longer agglutinated the donor's cells; all the agglutinin had been absorbed by the donor's transfused cells. On the other hand the patient's blood cells collected after the blood transfusion were agglutinated by his own serum saved from before the transfusion (because, of course, the patient's cells after the transfusion consisted largely of donor's cells, susceptible to patient's original agglutinin). These conditions persisted for several days in this case.

Human iso-agglutination seems to vary in intensity from time to time, but there are no accurate studies on this question, and the causes of the variation are unknown. (I can add from recent work with Dr. Friedman that rabbits' iso-agglutinins seem to be far less constant than those of dogs or human beings.)

THE FORMATION OF METASTASES AFTER AN INTRAVASCULAR INJECTION OF TUMOR EMULSIONS.

I. LEVIN, M.D., AND M. J. SITTENFIELD, M.D.

(*From the Department of Pathology of Columbia University, New York.*)
(George Crocker Special Research Fund.)

It is generally accepted that metastasis in malignant tumors is formed by the proliferation of the tumor cells which have been transported to distant parts of the organism through the blood or lymph channels. The cells of the primary tumor penetrate in some manner into the lumen of the vessels, are swept away by currents as emboli, and finding lodgment in some distant part of the organism, they proliferate and form secondary tumors.

This conception of the formation of metastasis was established through observation of autopsy material and no direct experimental proof of the matter was adduced up to the present. All experiments with intravascular injection of human tumor material into animals either gave negative results or were entirely untrustworthy.

In all the extensive literature of the last decade on the subject of the transplantable cancer of the white mouse and rat there appears no statement in regard to intravascular injection of tumor material with the aim of forming metastasis. The only exception is a short note by Graf, who obtained negative results.

Metastasis in malignant tumors of the white mouse and rat occurs rarely as compared with human cancer, and the channels for the transportation of the tumor cells are in the majority of cases the blood vessels. The reason for the rare occurrence of metastasis in the rat and mouse, Ehrlich, in accordance with his athreptic theory of immunity, sees in the fact that tumors in these animals are usually of extreme malignancy and grow to very large size. The cells of the primary tumor use up all the specific food found in the organism of the host, and the cells

transported from the primary tumor to other regions of the organism do not find the necessary nourishment and consequently cannot proliferate. Carl Lewin, on the other hand, thinks that the fact that metastasis in these animals takes place only through the aid of the blood vessels and not the lymphatics, accounts for the rare occurrence of metastasis in these animals. As was shown by M. B. Schmidt through his observations on human cancer, blood is capable in majority of cases of destroying cancer cells, found within the blood vessels.

The study of the influence of the blood upon the cancer cells carried by it and of the capacity of such cells to form metastasis may serve to clear up a number of phenomena in the genesis of cancer. In view of this a systematic study was undertaken by the writers on the experimental formation of metastasis, of which the present communication forms the first report.

The experiments consisted in an injection of a tumor emulsion into the jugular vein or carotid artery of a white rat, and were conducted on three different tumors.

Sarcoma of the white rat.—This tumor is very malignant, grows to a large size, and the occurrence of metastasis after a subcutaneous inoculation is very rare. In hundreds of animals inoculated by the writers, local dissemination was observed not more than in a half dozen cases, and once only was metastasis found in the liver. An emulsion of this tumor was prepared by cutting it in Haaland's mincing machine, grinding in normal salt solution and filtering through a layer of coarse gauze. The milky opalescent fluid contains a sufficient number of living cells to produce a tumor growth upon a subcutaneous inoculation. Thirty-six rats received an injection of this emulsion into the jugular vein. The animals were killed at periods ranging from eight days to four weeks after the injection and a thorough search made in all the organs for metastasis. Not in a single instance was a metastatic tumor found. No microscopical study was made of the organs which appeared normal on gross inspection, since this investigation does not concern itself with the question, where the tumor cells circulating in the blood find

lodgment, but whether such a cell transported into a certain organ will form there a visible metastasis. All suspicious nodules found anywhere and all lungs appearing abnormal on gross inspection were examined microscopically with negative results. The same negative results were obtained in six rats, where the injection was made into the carotid artery, and the animals survived. This method is very difficult of execution and the animals usually die a few minutes after the injection from respiratory paralysis, while the heart continues its action a few minutes longer.

Carcinoma of the white rat.—(Flexner-Jobling.) This tumor is not as malignant as the previous one, but metastasis occurs frequently after a subcutaneous inoculation. The metastasis is usually found in the lungs, the heart and even in the lymphatic glands, though the authors believe that in most cases the metastasis were produced through the blood current. Sixteen rats received an injection of an emulsion of this tumor into the jugular vein. In three animals metastasis was found in the lungs; no other organ showed any metastasis. Six rats survived an injection into the carotid, and of these animals one showed metastasis in the lungs. In another rat, in which the injection was made in the carotid against the stream of blood, the animal was found dead twelve days later. At the autopsy a nodule was found on the wall of the left ventricle. The animal remained dead in the cage over night, and the specimen had greatly deteriorated, still the nodule resembled microscopically the picture described by Flexner and Jobling for a metastasis found by them in the heart.

Sarcoma of the white mouse.—Ehrlich has shown that when this tumor is inoculated subcutaneously into a rat, there forms a small nodule, which remains for eight to ten days, and is then absorbed. No metastasis formation of this mouse tumor into a rat were ever noted. An emulsion of this tumor was injected into the jugular vein of twelve rats. The rats were killed in periods of four to eight days, and in two of the animals

metastatic nodules were found in the liver. Neither in the lungs nor in any other organ was there found any metastasis.

The results of these experiments seem to indicate, in accordance with the opinion of M. B. Schmidt, that cancer cells introduced into the blood circulation in a majority of cases lose their proliferating power. Ehrlich's opinion that the most virulent tumors do not form metastasis also seems to be correct, as no metastasis was formed upon the injection of the most virulent sarcoma of the rat. But the athreptic theory does not explain this fact. None of the animals had any sarcoma growth anywhere, and consequently the sarcoma cells introduced into the circulation could find all the necessary specific food.

The most interesting phenomenon observed in the course of these experiments consists of the fact that, while the Flexner tumor found lodgment upon an intravascular injection in the lungs, the mouse sarcoma produced metastasis only in the liver. It would seem that the different topographic distribution of metastasis in the different kinds of malignant tumors is due not so much to the difference in the channels through which the cancer cells are transported, and which were identical in all experiments reported here, but to a specific affinity between cancer cells and cells of certain organs. The comprehension of this specific affinity between a cancer cell and a particular part of the organism of the host may be helpful in elucidating many factors in the genesis of tumors and will be the main object of the further study of the experimental metastasis formation by the writers.

Discussion:

Dr. Moschcowitz thought that the paper of Dr. Levin was highly suggestive, but it did not explain why the involvement of lymphatics in carcinoma was nearly always reginal, nor could it explain such phenomena as retrograde lymphatic involvement. According to Dr. Levin, involvement of lymphatics would be a haphazard affair, and would not occur with the regional precision that it does.

Dr. Larkin: Goldman and Schmidt's research in cancer cells is a very interesting piece of work. I doubt, however, if the Goldman and Schmidt observations are universally concurred in. However, the mode of transportation of cancer cells, after all, is not of so very much importance in the formation of a metastasis as the factor that causes cell growth after it goes from an apparent focus to a distant part. This is something which in pathology we are always interested in.

However, Dr. Levin's presentation of the theory of Goldman and Schmidt was quite accurate indeed, and deserves a great deal of consideration in the elucidation of this problem of metastasis.

Dr. Pappenheimer: I would ask Dr. Levin if any experiments have been made with the direct injection of cancer extracts into different organs—and if by that method the organs show the same selective affinity for a given tumor as they do with the intravenous injection.

Dr. Levin: Neither Goldman nor Schmidt, much less myself, claim that blood vessels are the only channels of transportation of metastasis. There is no question that cancer cells may travel through lymphatics. The researches of Goldman and Schmidt are of great importance mainly for the reason that they indicate that the same channels may serve for the transportation of all kinds of tumors. The difference in the localization and character of metastatic tumors is due only to the difference in the affinity between organ cells and tumor cells.

To answer Dr. Pappenheimer, I may state that inoculations into parenchymatous organs, while presenting a number of interesting phenomena, do not help in the elucidation of the question of formation of metastasis.

DEMONSTRATION OF SPECIMEN: CHORIO-EPITHELIOMA OF THE UTERUS.

J. G. HOPKINS, M.D.

The patient was a woman of 33 years, who in last July was delivered of a six months infant which died shortly after birth. Past history was practically negative, there having been no illness since childhood, but a few weeks before delivery she had had some metrorrhagia and after delivery she continued to bleed and lost weight and strength. There was no examination made of the placenta at the time of miscarriage and we were unable to find out anything about its condition. She grew progressively weaker, and had one or two attacks of syncope. On September 11 she was admitted to the Sloane Hospital for Women on Dr. Cragin's service. She at that time had a cough with bloody expectoration which had already been present for two or three weeks. There was one nodule palpable in the vagina. On account of the evidence of quite extensive metastasis no operative interference was attempted. On October 3 she died, three months after delivery. From the time of admission she had a fever of a septic type, at one time as high as 105°.

At autopsy the uterus contained in the posterior wall a soft and necrotic mass, which extended out and penetrated the uterine muscle at the lower portion of the cul de sac. It also extended out through the broad ligament on one side and the uterosacral ligament on the other. There were three nodules in the vaginal wall. The lungs presented the striking and characteristic appearance often found in these cases; they were studded with hemorrhagic nodules from 0.3 to 2 cm. in diameter. The distribution of them was rather interesting; they were situated almost directly beneath the pleura like small infarcts. The only other metastasis was one small nodule in the liver. In the left renal vein there was a free thrombus about 3 cm. long, quite firm, but section of it showed no tumor cells. The right ovarian vein was much dilated and contained a thrombus, in which also no tumor cells were found. The ovaries were practically nor-

mal; they were not enlarged or cystic, and contained a few early corpora fibrosa.

Microscopically the growth did not show the typical appearance of a chorio-epithelioma. The primary tumor was so necrotic that very little could be determined as to its structure, but in the uterine muscle about it were many multi-nuclear and mono-nuclear giant cells, probably of syncytial origin. In some fields these were apparently invading the uterine vessels. The smaller metastases consisted of dense fibrin resembling canalized fibrin of the placenta, and in the periphery of nodules were giant cells similar to those found in the uterus. The larger metastases differed only in containing masses of blood, due apparently to secondary hemorrhage into the nodule. Occasionally a few cells with clear, pale protoplasm and large vesicular nuclei were found, which resembled the cells of Langhans, but none were found which were absolutely characteristic. Following Marchand's classification, this tumor would fall under the atypical chorio-epitheliomata, and according to Dr. Ewing's classification would be called probably a syncytioma. It would seem that curettings from such a uterus would be rather difficult to distinguish from the conditions which he classifies as syncytial-endometritis, though the nuclei are larger and more pyknotic than would be found in such a condition and might enable one to make the diagnosis of malignancy. Judging from the clinical course, a secondary sepsis may have had much to do with the fatal outcome, though it seems hardly possible that a patient could recover after such extensive metastasis. The gross appearance seems to indicate clearly the malignant character of the tumor.

Discussion:

Dr. Larkin: I have not had an opportunity of looking at the sections, but I am struck by the short duration of the case, which resembles in this respect a similar one which I have seen in which the pregnancy occurred in June and death took place in September.

I wish to comment upon one point in Dr. Hopkins' presentation—that is, the occurrence of giant cells beneath the peritoneum of Douglas' cul-de-sac. This is of importance, especially in inflammatory conditions of the uterus not connected at all with tumor growth. It may be of very great importance in the diagnosis of a preceding pregnancy, since it has been found that the large wandering giant cells may migrate out through the uterine wall and be found underneath the peritoneum in the cul-de-sac.

Some time ago we saw a specimen from a case of abortion where the only evidence of that abortion was the finding of giant cells right underneath the peritoneum. In fact, in cases of abortion, after complete curettage of the uterus, I have noted the presence of large giant cells deep down in the musculature, far distant from any point which could be reached by a curette.

INTERNAL HYDROCEPHALUS, WITH SPECIMENS SHOWING OCCLUSION OF AQUEDUCT OF SYLVIUS AND OF FOURTH VENTRICLE.

M. G. SCHLAPP AND J. B. GERE.

We wish to present to you this evening four cases of internal hydrocephalus. I do not wish to dwell upon the general symtomatology of internal hydrocephalus, but lay particular stress upon the pathological findings in these four cases. In two of these cases I wish, however, to present some clinical data which, in connection with the pathological findings, make them of more interest.

The material of the first of these cases was sent to me by Dr. Ernest Sachs, whom I wish to thank for the same. This case was a male child three and a half years old who, the mother stated, was born with a large head, but up to nine months pre-

vious to its death seemed otherwise perfectly well. Nine months previous to the child's entrance to the hospital, he had a fall, after which he was unconscious and vomited repeatedly. One month after this fall the child became unconscious and developed a well-defined epileptic attack. Afterwards the child complained much of headaches and had several epileptic seizures. Five months after the injury, the child began to walk with an uncertain gait, and this became so marked that he could not walk or stand without falling. Vomiting increased, the child developed attacks of temper, became very irritable and excessively nervous. A spastic condition of the legs and arms, the Babinsky reflex and other symptoms typical of hydrocephalus were present. An exploratory operation was performed on April 5, 1911, and at nine o'clock on April 6 the child died. Specimens were sent to me cut through the crura, showing a complete occlusion of the aqueduct of Sylvius. We must infer that this closure of the aqueduct of Sylvius existed from the earliest period in the development of the child's brain and the fall which the child had can be considered as an injury which had a tendency to increase the cerebro-spinal fluid in the lateral ventricles, which in turn caused a marked increase of the symptoms.

No. 2.—Male child five and one-half months old, from the service of Dr. Howland, Bellevue Hospital. Pathological material was kindly given us by Dr. Charles Norris. Mother states that she noticed no abnormality about the child until the second month of his life. Then her attention was called to the fact that the child was developing a large head. Shortly after this time the child began to have peculiar spasms, lasting about five minutes, during which time the arms and legs became very stiff. When the child was four months old, it was taken to a hospital in the city where a lumbar puncture was made and about a pint of cerebro-spinal fluid removed, after which the child improved considerably. This improvement, however, did not last more than a week or so, when again the symptoms grew worse and the child was brought to the hospital, March 13, and died on March 27. There is no family history of any kind pointing to a possible

etiological factor. The greatest circumference of head, 63 cm. Occipito-mental circumference, 61 cm. Occipito-frontal diameter, 22 cm. Occipito-mental diameter, 21 cm. Biparietal diameter, 17 cm. Bitemporal, 13½ cm. Sub-occipito-bregmatic, 18 cm. The specimens of this case show very clearly an occlusion of the aqueduct of Sylvius and one may see that this occlusion is brought about by a distinct proliferation of glia cells which have practically erased the contour of the central gray matter. There is a distinct increase of cells presenting the appearance of a glioma, and not only an increase of glia fibrils.

No. 3.—Female child two and a half years old, which was born with a large head and the extremities completely paralyzed. It has never been able to move them. She takes nourishment fairly well. On admission to the children's service at Bellevue Hospital, it was found that although there was complete paralysis of the extremities, the child, so far as could be determined, reacted to sensory irritations, particularly to the prick of a pin, by crying. The child was blind and did not seem to hear, but when the child cried or seemed to smile, the movements of the face were carried out with perfect co-ordination. This showed that co-ordinated movements of the facial muscles were possible through emotional stimulation. I show you here a photograph of the child's head with the head of a normal child beside it, and you see the very marked difference in size. At the autopsy it was found that this child had no cerebral cortex. The cerebellum was present, although quite small. The basilar ganglia were present, but were somewhat diminished in size. The cortex being only a thin membrane, is in some places translucent, in other places slightly thicker and showing microscopically a few irregularly shaped nerve cells. By these specimens you see that the pyramidal tracts were entirely missing on account of the absence of motor cortex. How can we account for the co-ordinate movements of the face, the central motor neurons being absent? It is a well-known fact that as we trace the phylogenetic development of the central nervous system from lower animals to higher animals, the function of motion is transferred from the basilar

ganglia to the motor cortex. If, for instance, the motor cortex of a dog or cat be removed, it is found that these animals are still capable of walking, although in a disturbed manner, showing that a center other than the motor center in the cerebral cortex still has some influence over the movements of the extremities. This center, no doubt, is situated somewhere in the basilar ganglia. If the motor cortex of the human being or the higher monkeys is removed, we find that the extremities are totally paralyzed. Here the motor function has been completely transferred from the basilar to the motor cortex. It seems, however, that this transference of the motor function has as yet not taken place completely in movements of the face, and particularly has it not taken place in *emotional* movements of the face, so that we can say that the emotional movements of the face are still to a certain extent vested in the phylogenetic earlier motor centers of the basilar ganglia. This case having no motor cortex in the cerebrum distinctly proves this conclusion.

In this case you also see that the aqueduct of Sylvius is completely closed.

No. 4.—A female child, six months old. The parents were alive and healthy, two brothers, twelve and nine years of age, were living and well. Two sisters, both delivered instrumentally, died about an hour after birth. Whether their heads were larger than normal could not be ascertained. Otherwise, the family history was negative.

The child was normally born, without instruments. At the age of six weeks it had "bowel trouble," accompanied by "sick spells," during which it would remain unconscious for a whole day at a time. At the age of three months, the child had convulsions with twitchings of her entire body lasting about five minutes. The bowels were very constipated. The mother thought that the child's head had always been large, and had noticed that one eye had "turned out" since birth. It had never attempted to raise its head, nor did it appear to see. However she took her feedings well, and was well nourished.

Occipito-frontal circumference, 19½ in.; occipito-bregmatic

circumference, 18¾ in.; fronto-mental, 19¾ in.; from the top of one mastoid to the other over the head, 15½ in. Anterior fontanelle open. The child had no teeth.

An operation was performed by Dr. John Hartwell, for the drainage of the ventricles of the brain into the peritoneal cavity. A description of the operation and a more detailed clinical report will be published elsewhere.*

At the autopsy there was found a very interesting condition, in that the whole fourth ventricle was completely obliterated by the proliferation of choroidal cells. We found, beginning at the lower end of the fourth ventricle, choroidal cells growing from a peduncle situated at the raphe of the medulla. As we ascend cephalically, these choroidal cells increase in quantity, and in the region of the sixth nucleus, they entirely fill the fourth ventricle. Here, as well as at other places, the choroidal cells invade the nerve tissue of the cerebellum and also of the medulla and pons, in typical neoplastic fashion. The aqueduct of Sylvius, throughout its course, is completely obliterated, not by choroidal cells, but by the proliferation of the surrounding glia cells. At some of the points where these choroidal cells have invaded the nerve tissue of the cerebellum and medulla, we find them arranging themselves in such a way as to form the walls of cysts and to take on the characteristic formation of ependyma cells. These choroidal cells are unquestionably spongioblastic cells derived from the same mother cells from which the glia as well as the ependyma cells develop.

A fuller description of these four cases will appear later.

*We are indebted to Dr. John Hartwell for the specimen.

A COMPARATIVE STUDY OF METHODS FOR STAINING THE CAPSULES OF BACTERIA.*

GEORGE BAEHR AND JOHN KANTOR.

In accordance with the fact that the various methods suggested in the past have differed from each other primarily in their respective methods of fixation, we have chosen for this study procedures which appeared to be representative of the different methods.† After heat fixation, as exemplified by the welch[1] and Hiss[2] methods, shrinkage and distortion of the capsules were usually observed, and not infrequently capsules were not demonstrable although by other procedures they could easily be obtained. The results obtained with the Welch method were as a rule more satisfactory than those with the Hiss, and this we interpret as being due to the use of glacial acetic acid in the former. Drying of the smear previous to fixation usually had a very deleterious effect upon the preservation of the capsule, and this accounts in part for the relative inferiority of the Welch, Hiss, Wadsworth[3] and Medalia[4] procedures.

Many of the methods studied leave the albuminous menstruum, with which the smear is made, as a more or less deeply stained background. The best examples of these are the osmic vapor method of Weidenreich[5-6] and the tannic acid method of Rosenow.[7] Aside from the fact that this prevents a clear definition of the capsular outline, it frequently results in the production of artefacts such as retraction zones, the edges of which may be sharply stained. Such pseudo-capsules we have observed with B. coli. B. typhosus and other non-encapsulated bacilli. We know from Rosenow's[8] work that the entire capsular substance is stained by his method and therefore when the retraction zone is wide one can easily see that it is transparent and unstained and rule it out as an artefact. But when the retraction zone is

*Work done under the tenure of the Eugene Meyer, Jr., Fellowship in the Pathological Laboratory of the Mount Sinai Hospital.

†The following is a preliminary communication. More complete details of the work will later be published elsewhere.

exceedingly narrow, the stained margin of the albuminous background may envelope the organism very closely, simulating a capsule almost exactly.

If the procedure described by Buerger[9] is carried out correctly, the fixative (Zenker minus acetic acid) applied to the smear at the moment when the edges begin to dry and left on for not longer than three to five seconds, the background is completely eliminated and the organisms and their capsules are well fixed. The capsular outline, in the case of the pneomococcus, for example, is stained intensely and the capsular material much less deeply than the outline.

We believe that we can prove that this increased depth of staining of the capsular margin, as obtained with the Buerger method, is not dependent upon an appreciable amount of shrinkage. It appears to be due, more probably, to an increase of the normal concentration of protein or other substance at the periphery of the capsule. Not only is it not a disadvantage, but it is an important factor in accentuating the morphological differences between the capsules of pneumococcus, the streptococcus and streptococcus mucosus. Buerger[10] has described a pneumococcus type of capsule, a streptococcus type, and a mucoid type which includes the streptococcus mucosus and b. mucosus capsulatus. Several observers[3-11] have emphatically denied the existence of such distinctive types. From our observations we were enabled to ascertain that in every instance the capsule staining method employed was the cause of their disagreement, for by using these methods we also could not obtain any morphological differences. Even if these so-called types are artefacts, as has been claimed, the fact that they are differential would warrant the use of any method which would demonstrate such differences. But that they are not artefacts is easily proven by examining the organisms unfixed and unstained, which is easily accomplished by a hanging drop made by using a one per cent. collargol suspension.

Besides preserving the type capsule, a good method must bring out the poorly developed capsules. With many of the pro-

cedures, for example the Welch, Hiss and Wadsworth, we frequently could not demonstrate these degenerate forms. The Buerger and Rosenow methods proved most satisfactory in this regard. The Rosenow, the osmic vapor and the other methods in which a uniform background is retained, on many occasions produced pictures with non-encapsulated organisms which were very confusing. On the other hand it has been our experience during this study that the Buerger method when executed properly never failed to show a capsule when one could be demonstrated by any other method, never produced anything around a non-encapsulated bacterium which could be easily mistaken for a capsule, and most uniformly and distinctly demonstrated the various types.

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Discussion:

Dr. Buerger: Dr. Baehr has covered this subject so thoroughly that I can add very little, my experience in capsule staining dating back six or seven years. However, there are a few things I might point out. There can be no question about the fact that the capsules, as they appear with the Zenker method, are not retraction zones. When, in a mixed culture, the field is absolutely clear and there are many other bacteria without capsules, we have sufficient ground for believing the capsules to be true ones and we need not dwell further on the detailed arguments. I think Dr. Baehr's point as to the value of a clear background is

very well taken. The reason for the failure by so many men to detect encapsulated organisms, is due to diffuse staining, and the absence of a clear field. In my experiments with the various fixatives, including osmic acid, Fleming's solution, and formalin, I came to the conclusion that Zenker was the best for the particular purpose in question.

The capsule of the pneumococcus is stained as a rule faintly or not at all. By stain, of course, I mean a complete readily recognizable staining of capsules, something that everyone would at once recognize as a tinctorial effect.

Dr. Libman: The question of the existence of a type pneumococcus capsule is not purely of academic interest, but is of diagnostic import. It is, in general, important for us to know as soon as possible exactly which organism is causing the infection. This is particularly true since the introduction of vaccine and serum therapy. In actual practice there are two conditions in which it is very valuable to know as soon as possible whether an invading organism is a pneumococcus or not. These two conditions are sinus thrombosis and pneumococcus peritonitis. In our experience we have not found any cases of sinus thrombosis due to the pneumococcus. If, therefore, in a case of otitic disease a pneumococcus is found in the blood culture, we would not be ready to diagnose infection of the sinus, and would therefore not make the same indication as we would were a streptococcus present.

There exist occasionally cases of so-called idiopathic streptococcus peritionitis. The cases of idiopathic pneumococcus peritonitis are much more frequent. When a surgeon is operating upon a case of peritonitis and cannot find a focus of entry, he can close the abdomen with much greater assurance if we report a pneumococcus in the exudate (even if he has not looked for the primary focus).

EXPERIMENTAL POLIOMYELITIS, PRODUCED IN MONKEYS FROM THE DUST OF THE SICKROOM.*

M. NEUSTAEDTER, M.D., PH.D., AND
WILLIAM C. THRO, A.M., M.D.

(From the Department of Clinical Pathology of the Cornell University Medical College.)

Struempell, in 1884, and Pierre Marie, in 1885, were the first to call attention to the infectious character of poliomyelitis or the Heine-Medin disease, as they called it. As civilization became more and more complex, and population more dense, the disease became more widespread and its epidemics assumed larger proportions; so that Wickmann, Harbitz, and Scheel, in 1903, were the first who made a thorough study of the epidemics in Sweden and Norway, confirming the infectious character of the disease.

Landsteiner and Popper, however, proved this by the epoch making inoculations of the emulsion of the brain and spinal cords of infected children, who had died from this disease, into monkeys, intraperitoneally, reproducing the exact type in these animals. Levaditi and Landsteiner then showed that the virus is filterable through a Berkfeld filter, a discovery without which our work would have been an impossibility.

Flexner and Lewis, Strauss and Huntoon on this continent, and Romer, Leiner and Wiesner, Krause and Meinecke abroad, in 1910 confirmed these experiments. Flexner and Lewis in addition found that the virus was excreted through the mucous membrane, and were able to transfer it from monkey to monkey, but the mode of infection, the source, and the manner of dissemination of this disease were still mysteries, so that we were not certain what preventive measures to adopt for its eradication.

On February 10, 1910, before the State Medical Society, Dr. Flexner said: "Up to recently we have had no satisfactory

*Reprinted from the *N. Y. Medical Journal*, Vol. XCIV, No. 17, p. 813, 1911.

knowledge of the causation of this very destructive disease. Indeed, our ignorance has been so profound that we had no knowledge as to how the poison gained access to the body, and we have, therefore, been unable and are still unable to carry out any efficient prophylaxis against the disease. In other words, the medical profession has been quite as helpless in respect to this disease as has been the laity." In September, 1910, he published an article in which he maintained that the nasopharynx might be the portal of entry, and, in an article published in August, 1911, he made the following statement: "Insect contamination with the virus would serve, were it proved not only to be an experimental possibility, but to occur in nature, to clear away any present apparent discrepancies in the epidemiology of the disease. In this connection it should be stated that not only does epidemic poliomyelitis spread over a wide territory, but its spread is not promiscuous, but along the routes of human travel. Therefore, insects that seek human habitations and routes of travel, that possess the power to migrate over a considerable territory, that affect all classes of society, that abound during the period of greatest prevalence of the disease, and that do not wholly disappear at any season, should be the first to come under suspicion."

Dr. J. M. Armstrong (*Pœdiatrics,* August, 1910) makes two significantly inconsistent statements: "During the epidemic in St. Paul, Minn., seventeen cases occurred, infected from one another by visiting relatives." And yet he continues in another paragraph: "After investigating the hypothesis"—not mentioning how—"that the cause of this disease was due to food infection, water, dust, presence of domestic animals and insects, etc., these theories were abandoned as inadequate, and we came to the conclusion that the causative agent was a facultative parasite which might lead a saprophytic, saprozoic existence in the body and that conditions of fatigue or unknown phenomena cause it to change from a feebly virulent agent to one of extreme toxicity."

Robert W. Lovett (*Pœdiatrics,* August, 1910, pp. 574, 575)

summarizes a report of 150 investigated cases of poliomyelitis which occurred in 142 families and concludes: "The evidence of communicability in our series of cases is a very important matter. We had instances of direct contagion from child to child, with an incubation period of one to fourteen days. We had a number of instances of what appeared to be indirect contagion by a healthy carrier, and finally we had eleven instances in the 150 cases where the disease followed intimate contact with persons with old infantile paralysis, often of many years' standing. Instances of what would appear to have been contagion occurred in thirty-five out of the 150 cases. Of the 150 patients, sixty-two were swimming or wading in stagnant water just before the onset." . . .

Paul Krause (*Deutsche medizinische Wochenschrift*, October 21, 1909) and others report cases, where several members of the same family were in several instances simultaneously affected. He concludes that the infection is through the alimentary canal and therefore through articles of diet.

F. Mueller (*Münchener medizinische Wochenschrift*, 1909) states that in the epidemic of Hesse-Nassau the disease showed a predilection for certain villages, streets, and groups of houses. There was no evidence that the infection was conveyed through food or vermin, but it was apparently transmitted from person to person.

These are the opinions held by those who ventured to give any. We have been unable to find any theory that would coincide with ours.

The theory, which we are now in position to prove, was based on the following facts:

1. The disease is eminently an infantile one, occurring in dry season.

2. The character of the neighborhood and of the living premises, where it occurs, plays no material part in the spread of the disease. In other words, it can occur anywhere.

3. Several children in the same family may be attacked successively. In a given house, children are attacked in differ-

ent families in fairly quick succession, and along lines of neighborly communication.

From these facts we have concluded that the virus lurks in the dust, and that it must be contagious.

In the early part of 1910 we began to collect sweepings of the rooms where cases of poliomyelitis occurred and to prepare them for injection into monkeys. Throughout our experiments we have used (*Macacus rhesus*) monkeys and for purposes of accuracy we shall speak of our experiments with monkeys Nos. 1, 2, 3, 4, 5, and 6.

In March, 1910, we collected sweepings from nineteen cases of poliomyelitis of from six to three months' standing after the onset of the paralysis. These sweepings were collected from rooms where the children were playing and sleeping, by cleaning the floor and the wooden trimmings around the walls, and were dried for days, then sifted and macerated in normal salt solution for a week, then filtered through paper and a Berkfeld filter. This filtrate was kept on ice for six days. On April 8, 1910, we injected monkey No. 1 under the dura through a trephine opening in the right frontal skull. On April 15, seven days later, the animal became quite sick, but completely recovered after three days. The spinal fluid which was withdrawn before injection was not examined and no spinal fluid was withdrawn at the time of illness. A few days later we prepared extracts in the crude manner described from nasal discharges of some of those patients from whom the sweepings were obtained and injected them intraspinally into monkey No. 1, but without noticeable result.

Toward the end of April we obtained through the courtesy of Dr. Strunsky a sample of sweepings from a case of six weeks' standing. This was also handled in the manner described, and on May 2, 1910, we injected monkey No. 2. We thought monkey No. 1 might be immune and were anxious to take every precaution possible not to confound negative results with positive failures. On May 6, four days after the injection, the monkey became very ill and both monkeys Nos. 1 and 2 were killed and

autopsies were performed. Macroscopically, monkey No. 1 was normal, but monkey No. 2 presented a hyperæmia of the vessels throughout the brain and spinal cord. Dr. Dunlap was kind enough to examine these cords and following is his report: "In monkey No. 2, in the neighborhood of several vessels of the pia, especially the veins of the posterior portion of the cord, there were collections of small dark staining nuclei of lymphoid cells; these undoubtedly indicate some sort of reaction, possibly an infection (?), but I was unable to discover anything to account for it. In the gray matter I found very little that I could recognize as pathological. In the specimen of the lower cervical region, as you will see, there are scattered extravasations in the gray matter, especially on one side; but this was the only level at which I found such changes. I thought the number of stellate cells might be slightly increased in both animals. Neuroglia increase is certainly slight, if present at all; I was not able to satisfy myself that there was any reaction.

"I do not know the conditions of your experiment, but I understood that the work was undertaken with a view to producing experimental poliomyelitis; if such is the case the cords in neither of these animals show any changes which I should consider in any way characteristic of that process, as it occurs in human spinal cords, the changes of monkey No. 1 being confined essentially to the meninges, more especially in the neighborhood of the blood vessels."

This report convinced us that we were following a faulty technique and in May, 1911, we began our experiments again.

May 16, 1911, we injected into monkey No. 3 an extract of sweepings from three different cases which occurred in one house. These were macerated with normal salt solution for a few days and filtered through a Berkfeld filter. The cases were of two days' and two weeks' standing, respectively. The spinal fluid was withdrawn prior to injecting into the animal and examined. The monkey was then etherized and trephined in the right frontal skull and injected through the brain into the ventricle with four cubic centimetres of the prepared filtrate. This

filtrate, as well as those injected into monkeys 1 and 2, were inoculated into ascitic broth media and subsequently proved sterile after a period of several days' incubation. Wtihin twenty-four hours after the injection, the monkey rallied nicely and remained well until May 23, when it fell quite ill. It kept to a corner in its cage, lying down most of the time. May 24, the spinal fluid was withdrawn and examined. On this day it was constantly lying flat on its abdomen and upon examination we found the patellar and plantar reflexes gone in the right hind limb. May 25, there was a partial paralysis of both hind limbs and on the day following all four extremities were paralyzed. There was also a rigidity of the muscles of the neck, and when placed on the dorsum, the animal could not turn. Pupils were normal and reacted well; there was no strabismus. The reflexes in the hind limbs were absent and on extending the limbs the animal winced, evidently from pain. The animal was killed, but the autopsy failed to show any meningitis or encephalitis. A blood clot 1 mm. by 3 mm. was present in the fourth ventricle. This time the spinal fluid had a lymphocytosis and a positive globulin reaction. Macroscopical and microscopical findings of monkey No. 3 were negative. We have, however, made an emulsion of its brain, cord, and ganglia for testing on the other monkey.

May 26, monkey No. 4 was injected intracerebrally with the emulsion of monkey No. 3, after first withdrawing the spinal fluid, and up to August 19 showed no reaction. On this day this same animal was again injected intracerebrally with an extract of sweepings of a case of one week standing which we received through the courtesy of Dr. Sayre on August 12. The extract was then prepared in the following manner: The sweepings were shaken constantly for six hours with 100 c.c. of normal salt solution and then strained through cotton; 30 c.c. of this filtrate were filtered through a Berkfeld filter and evaporated in a vacuum in the course of three days to 25 c.c. A pure culture of *Bacillus prodigiosus* was added to the extract prior to filtering it through the Berkfeld, in order to test the permeability of the filter. A subsequent inoculation of this filtrate into blood agar

proved it to be sterile. The possibility that *Bacillus prodigiosus* toxine may have had any effect upon the results is excluded by the fact that subsequently from monkey No. 5 the virus was transferred to monkeys Nos. 4 and 6 by the injection of an emulsion which was not passed through a filter.

The procedure was the following: Monkey No. 4 was tapped in the lumbar region and 4 c.c. of a clear fluid were obtained and examined for lymphocytes and globulin reaction. The globulin reaction, according to the Noguchi method, was negative. The monkey was etherized, and through a trephine opening in left frontal skull, 5 c.c. of prepared extract were injected through the brain substance into the left ventricle. Half an hour after the injection, the animal was up and about, and quite lively, and continued in an apparently good state of health up to August 26, seven days after the injection, when it became nervous, trembling at the least disturbance, dull, and listless and kept to a corner of the cage. On the following day, we noticed a flaccid paralysis of his right forepaw, a characteristic wrist drop, and a disappearance of the symptoms of malaise. His condition ever since remained normal with the exception of the paralysis. We did not kill this monkey, because the paralysis was rather insignificant, and we were doubtful of the results. August 30, a lumbar puncture was made, and one c.c. of a clear, spinal fluid was obtained and examined.

August 29, we obtained, through the courtesy of Dr. Wallrath, of Staten Island, the sweepings of a room wherein a four-year-old child was stricken with poliomyelitis, and had been then two days paralyzed. In this room the child passed most of its time, and was confined to bed.

The negative results in the first three monkeys, and the doubtful one in the last experiment on monkey No. 4 suggested that our technique was at fault, and we resolved to use sterile water instead of normal salt solution, and obtain the filtrate ready for injection at the earliest possible moment after the collection of the sweepings.

Accordingly, this new installment of dust, about fifty

grammes, was macerated over night with 30 c.c. of sterile water, and then shaken for one hour. The mixture was filtered through a paper filter, and 15 c.c. of this filtrate were passed through a special Bougé filter, under high pressure, within an hour.

Cultures of this filtrate were made on bouillon and blood agar. The cultures grew a Gram positive coccus within twenty-four hours, probably a subsequent contamination, for none was found in the animal after the autopsy.

Within eighteen hours after the collection of this dust, 5 c.c. of the filtrate were injected into monkey No. 5 through the spinal fluid, and 8 c.c. were injected subcutaneously.

After the injection the animal was depressed and sullen, and inattentive to its surroundings. On the following day the monkey was perfectly well and remained so until September 4, six days after the injection, when it was reported very ill and paralyzed. September 5, the animal presented a flaccid paralysis in the right foreleg, and a marked paresis of both hindlegs, most pronounced in the left. He was able only with great difficulty to move about in his cage. A lumbar puncture was made, but only two drops of a gelatinous material were obtained from the needle, after withdrawing it from the canal.

September 6, he still showed all symptoms mentioned, but in a more marked degree. A spinal puncture was again made, and two drops of a gelatinous material were obtained.

The animal was then killed and following are the results of the autopsy: There was a marked hyperæmia of the meninges in the lumbar region, and a lesser one in the cervical region; the vessels of the brain, cortex, and cord were markedly congested. This hyperæmia was evident at all the levels of the cord, in the medulla, in the pons, the ventricles, and basal ganglia. The cord and brain presented an œdematous condition. On section, one could note hæmorrhages into the gray substance of the cord, especially in the lumbar and cervical regions. There were also some hæmorrhagic foci in the medulla, while the white and gray substance of the brain presented, macroscopically, a normal appearance. The vessels in the ventricles were very much con-

gested. The viscera presented the following changes: Areas of congestion in the middle and lower part of the upper and the lower lobes of the left lung. The spleen was firm and congested, but not enlarged; all the visceral vessels were very much injected. All other organs and glands were, to all appearance, normal.

Sections of the spinal cord, cerebellum, pons, medulla, brain, spinal and basal ganglia were now emulsified in 50 c.c. of normal salt solution and preserved for injections. We wanted first to await the histological findings before injecting another animal. For diagnostic purposes a few sections were hastily hardened and stained.

The sections presented the following pathological changes (the lumbar and cervical sections alone being examined under the microscope):

There was a diffuse infiltration of round cells into the pia and gray substance of the cord, both in the anterior and posterior horns, but more so in the anterior. From accompanying photomicrographs it can be seen that the inflammation was greatest in the anterior part of the cord, following the pial processes into the depth of the anterior fissure and along the sheaths of the central vessels. The congestion in the gray matter was quite extensive, as evident by the engorgement of the capillaries. We further noted a circumvascular and interstitial round cell infiltration of two types of round cells, characteristic of poliomyelitis, also endothelial and polymorphonuclear cells. The ganglion cells began to show degenerative changes. There was also a marked gliosis, an additional evidence of the disease. We call attention to some features in order to leave the least possible doubt as to our success. Cultures made from the brain and cord of monkey No. 5 on the date it was killed proved negative. The autopsy revealed no *purulent* meningitis, but rather a hyperæmia of the lumbar, and cervical meninges. In examining the fluid drawn from the spinal canal on the day before the animal was killed, and on the day of making the autopsy, no bacteria were seen.

Our diagnosis having been confirmed on September 8, we

TABULATED RESULT OF THE EXAMINATION OF THE SPINAL FLUIDS OF MONKEYS NOS. 3, 4, 5, AND 6.

	Date.	Amount.	Clearness.	Color.	Coagulum.	Sediment.	R. B. C.	W. B. C.	Ratio.	No. in one cubic millimetre.	Multinuclears.	Small uninuclears.	Large uninuclears.	Transitionals.	Endothelial.	Bacteria.	Cultures.
Monkey No. 3, before injection	1911 May 16	gtt. x	Clear	None	None	None	....	..	....	3	..	..	..	..	..	0	0
Monkey No. 3, paralyzed.....	May 24	1 c.c.	Clear	Blood tinged	None	Slight amount	15	23	....	230	2	96	..	..	1	0	0
Monkey No. 4, before injection	April 27	4 c.c.	Clear	None	None	None	....	..	....	0	..	2	..	..	..	..	..
Monkey No. 4, paralyzed.....	August 30	1 c.c.	Blood tinged	Yellow	None	Reddish	374	2	1:185	46	4	17	5[1]	..	1	0	..
Monkey No. 5, before injection	August 31	5 c.c.	Clear	None	None	None	47[1]	9	1:5.2	44[1]	10	16	3[1]	0	0	0	..
Monkey No. 5, paralyzed.....	September 5	gtt. ii	Blood tinged	Red	Gelatinous	None	Many[1]	..	....	..	18[1]	40	6[1]	4[1]	0	0	..
Monkey No. 5, paralyzed.....	September 6	gtt. ii	Cloudy	Red	Gelatinous	None	Many[1]	..	....	..	46	18	28[1]	8[1]	0	0	0
Monkey No. 6, before injection	September 8	3 c.c.	Clear	None	None	None	2	4	1:2	4	..	3[2]	1[1]	0	0	0	..
Monkey No. 6, paralyzed.....	September 22	gtt. ii	Blood tinged	Bloody	.....	.....	130	0	....	..	6	4	8	..	..	0	0

[1]Blood present in fluid.

[2]Gay and Lucas, article in *Internal Medicine*, vi, 330-338, 1910.

injected monkeys No. 4 and No. 6, each with 6 c.c. of the preserved emulsion, intraspinally, and 8 c.c. subcutaneously.

Monkey No. 4, which we concluded might have had a poliomyelitis, immediately after the injection was thrown into a violent tremor and became quite ill. He could hardly be aroused and remained motionless for the rest of the day. The next morning he completely recoveerd. This was probably an instance of anaphylaxis due to the previous cord emulsion. On September 14, six days after the injection, this monkey became very ill and on the following day the right fore limbs and hind limbs became paralyzed. On September 16, eight days after the injection with emulsion of monkey No. 5, all four extremities were involved. The paralysis was of the flaccid type. Soon the muscles of the back and neck became affected so that the animal was unable to move even its head, and had to be fed. The reflexes were all gone and there was no response to the faradaic current in any of the extremities. Monkey No. 4 then reacted positively and thus proved that monkey No. 5 had poliomyelitis. It died on September 21 and the autopsy was made the same day.

Monkey No. 6, which was also injected with an emulsion of monkey No. 5, was quite ill on September 15, seven days after, but seemed to have been able to move about freely. It remained in this condition till September 22, fourteen days after the injection, when suddenly a flaccid paraplegia developed, and on the following day a complete paralysis of all four extremities. The monkey was then killed and an autopsy was performed.

Thus we had infected two monkeys from monkey No. 5.

In order to make the work more comprehensive, we may propose the schematic table found on this page.

The autopsy findings of monkeys Nos. 4 and 6 were as follows: No meningitis of either brain or spinal cord; the œdema of the entire cerebrospinal axis was very marked; the vessels were very much injected; on section there were punctate hæmorrhages at all the levels of the cord, medulla, and pons. In monkey No. 6 there were some adhesions of the spinal dura at

the lumbar and cervical levels. The visceral vessels were congested, but the viscera were all normal in appearance.

Microscopically, as seen in the photomicrographs, the sections present the same characteristics as those of monkey No. 5, with the exception that they are rather more intense, especially so in the lumbar region of monkey No. 6.

Cultures made from the ventricles and subdural spaces of brain and cord on ascitic broth and blood agar media, proved negative and no bacteria were seen on sections.

We have thus proved our theory that:

1. Poliomyelitis is propagated by dust, and we may also conclude that

2, the nasopharynx probably is the point of entry.

3. Acute poliomyelitis is both infectious and contagious.

It becomes perfectly evident that prophylactic measures should be most rigidly carried out in order to check the spread of this disease.

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DEMONSTRATION OF SPECIMENS.

DR. J. H. LARKIN.

(*a*) *Melano-Sarcoma.*—The first case is a case of Melano-Sarcoma of the Liver, taken from an individual about 64 years of age, who had his eye removed about one and a half years ago at the New York Eye and Ear Infirmary. After the removal of his eye, he commenced to undergo progressive emaciation with enlargement of the abdomen. Finally he was taken to Bellevue, where he remained for some time, the diagnosis there being Cirrhosis of Liver. After remaining perhaps a week or more, he was sent over to the Island; there he remained about three weeks. The history having been gone over and the absence of his eye having been inquired into, we obtained possession of the eye, which was removed one and a half years previously, and, on examining it, found a melano-sarcoma of the choroid. He died four days thereafter, the status at that time being as follows: There was marked cachexia, marked distention of the abdomen and pigmentation of the skin. No metastases were present in the bones or subcutaneous tissues.

At autopsy the particular feature of interest was the condition of the liver, which weighed 22¼ pounds. I present this specimen to-night as a very beautiful specimen of a Melano-Sarcoma; it shows a peculiarly interesting feature, that is, the transition in the type of the nodular or metastatic masses in the liver, which exhibit every gradation in size and in color, from a pearl white to a dense black. The only metastatic nodules in this case were found in the spleen, one medium sized nodule and several small ones. The urine obtained during life gave a pronounced melanin reaction, although this is now held to be of questionable clinical significance.

(*b*) *Acute Endocarditis due to an intermediate Diplococcus.*—The next specimen I have to show is one of some interest, and I must ask the indulgence of the Society for showing what at first sight may be considered a simple case of malignant endocarditis.

The specimens were taken from a young girl who was admitted to the City Hospital on August 29 and died September 28. She was about thirteen years of age, but she looked as if she was about eight years old. The history of this child briefly is as follows: Patient had scarlet fever about eighteen months ago, preceded by otitis, which had lasted for five or six years. Ten days before admission she began to complain of headache and chills. The physician called in detected a heart murmur, and as she had a temperature of 103°-104°, sent her to the Harlem Hospital, from which she was transferred to the City Hospital. She remained under observation until September 5, when her temperature ranged around 104°-105°, and at that time she had a very marked discharge from the ear, with some pain over the mastoid. She was operated on for a mastoid suppuration, and no great amount of pus was found. Her temperature ranged around 103°-104° and still kept going up. Four negative blood cultures were obtained; five days before death, however, there was obtained from the blood a diplococcus intermediate in type between streptococci and pneumococci. This organism, so far as it has been studied, corresponds morphologically and culturally with the diplococcus described by Rosenau, Libman and Celler in cases of endocarditis running a sub-acute course. If I remember rightly, in all the cases associated with this type of organism there have been old valvular or endocarditic lesions; this is apparently the first case which has occurred in a heart previously normal. The organism is not pathogenic for the laboratory animals, does not have any tendency to arrange itself in chains, and produces precipitation in serum-glucose agar. It is a non-capsulated diplococcus.

Petechial spots were found in the spleen, kidney and mucous membranes, but there was nowhere any abscess formed, and only one small infarct in the kidney. This seems to me of importance in differentiating a case of this sort from an ordinary streptococcus infection. In addition to the vegetations on the aortic valve, a number of anomalies were found. There were only two aortic cusps; the uterus was of the marsupial

type with complete absence of the Fallopian tube on the right side. The ovaries on both sides were normal. There was oedema of the left lower extremity, due to a white thrombus of the left iliac vein.

Discussion:

Dr. Libman: I do not want to go into details just at the present time concerning the organisms found in these cases. When our paper was published, we left the question open as to whether the organisms usually found were changed pneumococci or changed streptococci. In previous studies made in the laboratory, we had often found that pneumococci change partially or almost completely into streptococci, but had never found the reverse to be true. From our experience, therefore, it would have been more natural to surmise that these organisms were altered pneumococci. Dr. Celler and I were, however, never successful in obtaining capsules in these organisms and, furthermore, cultures of these organisms were not dissolved by bile, as pneumococci are.

In our paper we stated that we could surely regard these organisms as altered pneumococci if we found any pneumococci which on cultivation from the body could be dissolved in bile, but which on later cultivation from the body were found insoluble in bile.

When we read Dr. Rosenow's paper, we obtained the impression that he found capsules on these organisms in the primary cultures from the body, and these capsules being of the type described by Buerger as pathogonomonic with the pneumococcus. We have obtained a type capsule hitherto on only one organism on direct cultivation from the body. As we have believed in the pathognomonic value of the type capsule, we must regard this particular organism as being a changed pneumococcus. Because of the apparent discrepancy of our results and those of Dr. Rosenow, I suggested to Dr. Baehr to make a comparative study of the various methods of staining capsules, in order to determine whether or not we were missing capsules

on these cocci, due to the fact that we were using a method which would not give results as often as other methods might. As he will state to you in his report, he found that the method that we used gives capsules more regularity and better than any of the methods which he tried. Meanwhile, in a personal conversation with Dr. Rosenow, I learned that he obtained capsules only after repeated inoculations into rabbits, and then only with some of the organisms. He did not at any time obtain a capsule from a freshly isolated organism.

It is to be noted here that cases of endocarditis showing similar cardiac lesions to those shown by Dr. Larkin to-night, and the same clinical picture may be caused by the influenza bacillus. In the last few years certain evidence has accumulated which goes to show that the lesions in patients suffering from chronic infective endocarditis (or subacute bacterial endocarditis, as I prefer to call it) may undergo healing in part or entirely. In a study of our cases we have noticed a marked tendency to healing in some cases and, in a few, complete healing. Complete healing of the lesions, however, does not always go hand in hand with clinical recovery. The kidney lesions of this disease, as pointed out by Loehlein, appear to be quite characteristic. They are at present being studied in our laboratory and will be reported upon by Dr. Baehr.